

F109 Inhibition of Telomerase and Induction of Apoptosis in Human Cancer Cell lines by Green Tea Epigallocatechin gallate

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Tea is one of the most common beverages consumed worldwide, and the possible beneficial health effects have received a great deal of attention. Recently, several lines of evidence on the anticancer activities of tea catechins have emerged from animal models and human epidemiological studies. However, the exact mechanisms underlying these activities are still elusive and mostly speculative. In this study, we investigated the effects of (-)-epigallocatechin gallate(EGCG), the main constituent of green tea, on the induction of apoptosis(programmed cell death) and the inhibition of telomerase activity using five human cancer cell lines. Telomerase activities were detected by TRAP assay in cells treated with EGCG(15uM) and untreated. To ascertain whether EGCG can induce the apoptosis mediated by p21, we evaluated the EGCG effects in p21-supressed and nonsupressed cell lines. Through this study, we expect to explain the mechanisms underlying the anticancer effects of tea.

F110 Characterization of Mud Loach β -Actin Gene Regulatory Regions and Construction of Useful Fish Expression Vectors

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Transgenic fishes are of interest for basic biological studies and for genetic improvement of aquaculture fishes. Mud loach (*Misgurnus mizolepis*) has attracted attention as useful materials not only for commercial value, but also for investigating molecular interactions that occur during vertebrate development. Generation of the genetically engineered mud loach is a potential tool for such studies. For genetic engineering of fish, suitable expression vectors are required. Accordingly, we developed fish expression vectors, which contain 4.3 Kb upstream sequences of translation initiation of the mud loach β -actin gene. But these regions induced too much strong expression of the linked gene in transgenic mud loach. Therefore, we decided to analyze the regulatory regions mud loach β -actin gene in an attempt to generate transgenic fishes where the level of linked gene expression is modulated at will by juxtaposing the proper regulatory region of β -actin gene. To analyze the transcriptional regulatory regions, upstream 4.3 Kb of translation start codon was subcloned into pBS vector, and then juxtaposed to jelly fish green fluorescent protein (GFP) gene, as a reporter. We then made a series of deletion mutants within upstream regions and/or putative enhancer in first intron in combination. Transcriptional activity of each mutants was examined by transient transfection into fish cell lines PLHC or monkey kidney E25B2 cells, and then monitored the GFP expression by positive cell counting in fluorescence microscope or RT-PCR assay. We certified that the proximal promoter and conserved sequences in first intron are positive regulatory elements in both transcription rate and probability. Furthermore, we found that upstream sequences of proximal promoter act negatively in this assay. Based on these facts, we propose a suitable regulatory regions of β -actin gene useful in transgenic fish.